

Genome Sequence of the Haloalkaliphilic Methanotrophic Bacterium *Methylobacterium alcaliphilum* 20Z

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***Methylobacterium* strains are widespread in saline environments. Here, we report the complete genome sequence of *Methylobacterium alcaliphilum* 20Z, a haloalkaliphilic methanotrophic bacterium, which will provide the basis for detailed characterization of the core pathways of both single-carbon metabolism and responses to osmotic and high-pH stresses. Final assembly of the genome sequence revealed that this bacterium contains a 128-kb plasmid, making *M. alcaliphilum* 20Z the first methanotrophic bacterium of known genome sequence for which a plasmid has been reported.**

Aerobic methanotrophy, widespread within the bacterial world, is commonly found in *Alphaproteobacteria*, *Gamma-proteobacteria*, and *Verrucomicrobia* (3, 8, 10). *Methylobacterium alcaliphilum* 20Z represents a methanotrophic clade adapted to high-pH and high-salinity ecosystems (4, 6, 13).

The assembled genome sequence of *M. alcaliphilum* 20Z was determined at Genoscope. A mate-paired GSflx 454 library with 3-kb insert size was constructed (22 Mbp; \approx 4.6-fold coverage) and combined with 454 Titanium reads (71 Mbp; \approx 15-fold coverage) for assembly using Newbler (version 2.0.01.14) with validation via the Consed interface (www.phrap.org). For gap closure, 1,163 PCRs between contigs were performed and sequenced. Potential base errors were corrected by mapping a total of 1,349,122 Illumina 36-bp reads onto the whole genome sequence using SOAP (<http://soap.genomics.org.cn>) as described previously (1) to finalize the 4.8-Mb assembly. Sequence annotation and comparative genome analysis are under way using the MicroScope platform at Genoscope (14).

M. alcaliphilum 20Z features a relatively large circular chromosome of 4.67 Mb, with an average GC content of 48.75%, three complete rRNA operons, 44 tRNAs, and 4,083 predicted protein-coding sequences in the initial annotation. The genome also features a circular plasmid of 128 kb of very similar GC content (48.70%) encoding putative systems for replication and conjugation transfer. To our knowledge, this is the first reported plasmid for a methanotrophic bacterium with a known genome sequence.

The C₁ metabolism gene inventory in *M. alcaliphilum* 20Z includes a single copy of the membrane-associated methane monooxygenase gene cluster (*pmoCAB*) (12), pyrroloquinoline quinone (PQQ)-dependent methanol dehydrogenase (*mxoA*) and an associated cytochrome *c*, genes for MDH assembly and PQQ biosynthesis, tetrahydromethanopterin- and tetrahydrofolate-linked C₁ transfer pathways, aldehyde ferredoxin oxidoreductase (*aorAB*), membrane-associated quinoprotein formaldehyde dehydrogenase (*adh*), two formate dehydrogenases, and the ribulose monophosphate (RuMP) pathway. Although serine cycle enzyme

activities have not been previously detected in strain 20Z (6), genes encoding *glyA*, *sga*, *hpr*, *gck*, *mclA*, and *mtkAB* are present. Key enzymes of the Calvin-Benson-Bassham cycle (5) are missing. Complete sets of genes for function of the Embden-Meyerhof-Parnas pathway, the Entner-Doudoroff pathway, and the pentose phosphate pathway were identified. Similar to other type I methanotrophs (11, 15), the genome encodes all enzymes of the citric acid cycle.

Nitrogen metabolism involves the gene inventory for urea uptake and hydrolysis, nitrate/nitrite reduction, and direct ammonium uptake, as well as glutamine synthetase/glutamate synthase and glutamate and alanine dehydrogenases for ammonium assimilation (7). A gene homologous to hydroxylamine oxidoreductase (*hao*), possibly handling hydroxylamine toxicity arising from oxidation of ammonia by pMMO, is present (2).

The genome sequence confirms that *M. alcaliphilum* 20Z has an ectoine biosynthesis *ectABCask* operon (9). Accumulation of sucrose is encoded by an *sps-spp-fruK-amy* gene cluster, for sucrose phosphate synthase/phosphatase, fructokinase, and a specific amylosucrase, respectively.

Availability of the genome of *M. alcaliphilum* 20Z greatly expands the use of molecular approaches for experimental and biotechnological investigations of this strain.

Nucleotide sequence accession numbers. The *M. alcaliphilum* 20Z genome sequence was deposited in GENEMBL under accession numbers [FO082060](#) and [FO082061](#) (project ID [73721](#)).

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